a confined environment, what are the cell tension fields in the epithelial monolayer under these conditions, how the cells adapt to the pressure and the shape and rigidity of the confinement, and how the whole monolayer react to the accumulated lateral pressure due to cell proliferation. These will let me decipher how epithelium deformation is driven by cell proliferation and tension during development.

3967-Pos Board B695

Mitotic Cell Shape - RNA Interference Screening for Genes Involved in Mechanics using Atomic Force Microscopy

Cédric J. Cattin[1], Martin P. Stewart[1], Yusuke Toyoda[2], Ina Poser[2], Frank Buchholz[3], Anthony A. Hyman[2], Daniel J. Müller[1].
[1]ETH Zürich, Basel, Switzerland, [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, [3]UCC, Medical Systems Biology, Medical Faculty, Technical University Dresden, Dresden, Germany.

Cell division in animal cells requires major changes in the cytoskeletal arrangement to achieve accurate positioning of the mitotic spindle and subsequent correct chromosome segregation. To this end, cells undergo drastic shape changes in a process termed mitotic cell rounding. The mechanisms controlling this process are not fully understood. Using an atomic force microscopy (AFM)-based assay that quantifies cell mechanical parameters in conjunction with RNAi gene silencing, we screened almost 1000 genes for their role in mitotic cell mechanics. We find ~5 % of the genes screened to strongly influence mitotic cell mechanics and provide results from experiments further investigating the role of a subset of these genes formerly not known to be involved in this process. We believe that mechanical phenotyping like presented in this study can lead to new and quantitative insights into the way cells regulate and maintain their shape and mechanical integrity.

3968-Pos Board B696

Measuring Actomyosin Function in a Living Parasite using a Laser Trap

Rachel V. Stadler[1], Lauren A. White[1], Brian P. Helmke[1], Ke Hu[2], **William H. Guilford**[1].
[1]University of Virginia, Charlottesville, VA, USA, [2]Indiana University, Bloomington, Bloomington, IN, USA.

The pathogenic parasite Toxoplasma gondii invades host cells as part of its life cycle. Forward motion of the parasite during invasion is driven by rearward motion of adhesion receptors through the parasitic plasma membrane. These adhesion receptors are coupled to a unique actin (TgACT1) that forms very short filaments (~100 nm) for which there is no structure to lend them polarity. These are driven rearward by a fast, single-headed, class XIV myosin (MyoA) that is attached to an inner membrane complex. It is unknown how this system of un-oriented, short actin filaments and membrane-associated myosin can result in directional motility. Here we show that the motile apparatus of Toxoplasma is not pre-organized for directional motion, but rather becomes directional after a period of randomly oriented force generation. A laser trap was used to position microspheres on live Toxoplasma and to measure the transduction of force from TgACT1 and MyoA through cell surface adhesion receptors. We found that a ~50 second period of randomly oriented bead movement was followed by the force becoming oriented toward the rear (basal end) of the cell. The stall force was only 5.6 pN, and we see occasional series of 5 nm steps that may represent the activity of single MyoA. Force becomes directional at the basal end of the cell approximately 3 seconds later than at the apical end of the cell, but the magnitude of force generation was independent of location on the parasite. Addition of the actin filament stabilizer jasplakinolide abrogated directionality. These data suggest that MyoA is activated in Toxoplasma soon after receptor ligation, but that actin filament dynamics are critical to direction finding, and consequently to the regulation of host cell invasion.

3969-Pos Board B697

Probing Forces on Newly Generated Spindle Microtubule Minus-Ends

Christina L. Hueschen, Mary W. Elting, Dylan B. Udy, Sophie Dumont.
UCSF, San Francisco, CA, USA.

The mitotic spindle is a dynamic self-organizing machine that coordinates cell division and preserves genomic stability. The ability to focus microtubule minus-ends into poles is crucial to spindle structure and function. However, our understanding of pole-focusing forces has been limited by the challenges of labeling and imaging microtubule minus-ends in established spindles. Here, we used laser ablation to sever kinetochore-fiber microtubules in mammalian cells and probe how the cell detects and organizes newly generated microtubule minus-ends. Within a few seconds of ablation, the cell recognizes new minus-ends and begins pulling them poleward. These pole-focusing forces exist throughout metaphase and anaphase and can move chromosomes rapidly, dominating other spindle forces. Opposing forces on chromosomes from the other half-spindle are able to slow, though not stop, the pole-focusing response, as indicated by faster pole-focusing speeds in monopolar spindles and during anaphase than in metaphase bipolar spindles. Together, our data indicate that microtubule minus-end focusing forces operate broadly and rapidly and are of similar magnitude to other spindle forces. These pole-focusing forces are thus well-suited to robustly maintain spindle structural integrity despite rapid turnover of spindle components and mechanical challenges.

3970-Pos Board B698

Changes in Mechanical Properties of Actin Filaments of Astrocytes After Invasion by Trypanosoma Cruzi

Gerson F. Cote-Flórez, Juan Camilo Vargas-Zambrano, John Mario González, Manu Forero-Shelton.
Universidad de los Andes, Bogota, Colombia.

Astrocytes are the most abundant glia in the central nervous system and are responsible for neuronal protection and ion homeostasis, among others functions. Given its importance several atomic force microscopy studies have analyzed their topography and rigidity and found that actin filaments can be revealed by rigidity maps. Likewise, some studies found that the Young's modulus (modulus of elasticity) can decrease in astrocytes in the presence of drugs, especially actin synthesis inhibitors. However, no studies have reported how the stiffness of these cells can change during parasite invasion. In the present work, we determine the changes in the Young's modulus of astrocytes after infection by Trypanosoma cruzi using Atomic Force Microscopy. T. cruzi is a highly infective parasite that is responsible for Chagas disease, considered a neglected tropical disease, which can compromise the central nervous system during acute onset or in immunosuppressed individuals. In particular, we analyzed the topography and elasticity maps of the astrocytes, before and after infection. We find that the parasitic invasion significantly decreases cell and filament stiffness.

3971-Pos Board B699

Single Cell Measurements of Intracellular Signalling, and Motility, in Macrophage Cells Sensing a Bacterial Infection

Eugenia Cammarota[1], Jiro Sakai[2], Clare Bryant[2], Pietro Cicuta[1].
[1]Cavendish Laboratory, University of Cambridge, Cambridge, United Kingdom, [2]Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom.

Macrophages are cells of the innate immune system in vertebrates. They are a cell type able to eat colloidal scale particles. In particular, these cells take up pathogenic bacteria, contributing to controlling a bacterial infection, and also acting to alarm the immune system and begin an inflammatory response.
Macrophages are motile, move around tissues. When the presence of pathogens is detected, a complex network of signal pathways is triggered; in this particular state the macrophage is said to be "activated". The aim of our research is the characterization of the activation process that takes place in macrophages on sensing the Salmonella bacteria. Working with cell culture systems, and exposing cells both to living bacteria, and to components of the bacteria, we investigate both the intracellular signalling, which feeds from a membrane receptor into the NF-kB signalling pathway, and also the phenotypic changes in cell motility and morphology.
Regarding the intracellular signalling, we make use of cell lines in which two key components are fluorescently labelled, and through a robust image segmentation routine we detect NF-kB translocation within the cell (repeated cycles of cytoplasm to nucleus), at single cell level. This allows us to quantitatively measure the intracellular variability, and to identify commonalities and differences within the clonal population.
Cell motility and shape are also affected by the detection of bacteria in the system: we quantify the migratory behaviour of macrophages, and how it changes depending on the different activation agents, linking the observations to putative biological functions. Experiments have been carried out observing cells behaviour after stimulation with Salmonella LPS (LPS is a molecule present on the outer membrane of gram-negative bacteria) and Interferon-γ (IFN-γ is a cytokine, expressed by macrophages as an intercellular signal).

3972-Pos Board B700

Traction Stress Dynamics During Chemotactic Amoeboid Cell Migration

Effie Bastounis, Reudi Meili, Begoña Álvarez-González, Juan Carlos del Álamo, Juan Lasheras, Richard Firtel.
UCSD, La Jolla, CA, USA.

Chemotaxis is involved in a broad range of biological phenomena such as during cancer metastasis. It requires a tightly regulated, spatiotemporal coordination of underlying biochemical processes that impact the mechanics of cell migration. In response to intrinsic and environmental cues, motile cells can adapt their migration effectively. Yet both the mechanisms by which this adaptation occurs and the role of the interactions between biochemistry and mechanics of cell migration are largely unknown.